# Quality control of overexpressed membrane proteins

Geertsma, Eric R.; Groeneveld, Maarten; Slotboom, Dirk-Jan; Poolman, Bert

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# Supporting Information

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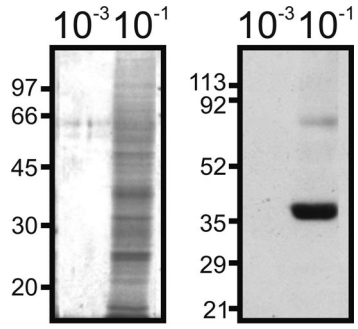


**Fig. S1.** Insoluble inclusion bodies isolated from cells expressing LacS to different levels. Expression of LacS by *Escherichia coli* MC1061 cells was induced with the percentages of L-arabinose indicated above the panels. Molecular masses (in kDa) and positions of the marker proteins are indicated on the left of each panel. (*Left*) Coomassie-stained gel. (*Right*) Immunoblot decorated with anti-His tag antibody.

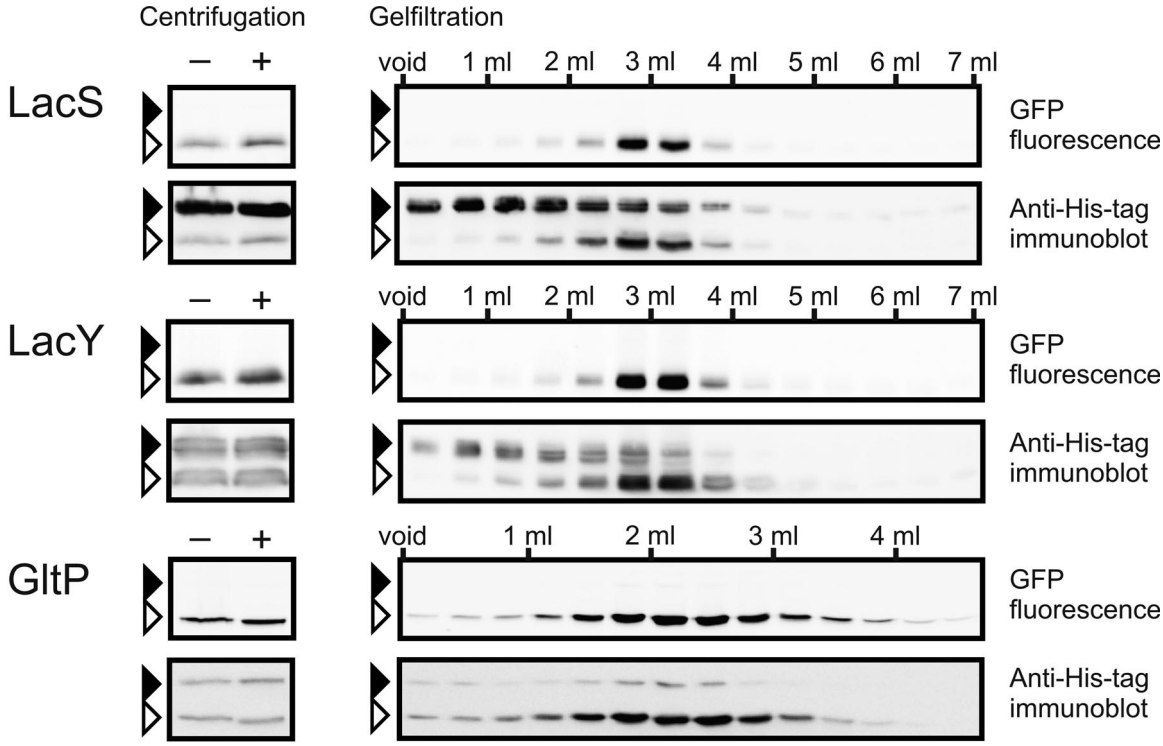


**Fig. S2.** Size-exclusion chromatography (SEC) on FC-12-solubilized cells. *E. coli* MC1061 cells were induced with $1 \times 10^{-1}$% (wt/vol) L-arabinose for 4 h at 25°C (LacS and LacY), and *E. coli* C43 cells were induced with 0.4 mM IPTG for 16 h at 17°C (GltP). Disrupted cells were solubilized with 1% (wt/vol) FC-12, and after ultracentrifugation the supernatant was analyzed by SEC. Samples taken before (−) and after (+) ultracentrifugation, and relevant fractions from the SEC were analyzed by *in gel* fluorescence and immunodetection using an anti-His tag antibody. Black and white arrows indicate the positions of nonfluorescent and fluorescent species of the GFP fusion proteins, respectively.

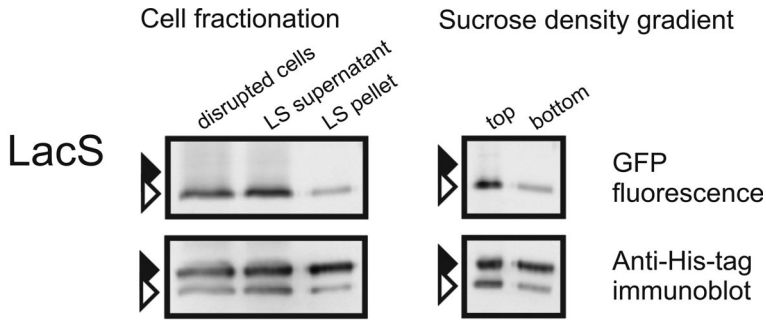


**Fig. S3.** Isolation of membrane vesicles. *E. coli* MC1061 cells were induced with $1 \times 10^{-1}$% (wt/vol) L-arabinose for 4 h at 25°C to allow expression of LacS. Cells were disrupted by two passes through a French pressure cell. (*Left*) The disrupted cells before centrifugation and the supernatant (LS supernatant) and pellet (LS pellet) after fractionation by low-speed centrifugation were analyzed. (*Right*) The supernatant of the low-speed centrifugation was further fractionated by sucrose density centrifugation. The top (low density; 5 ml) and bottom (high density; 1 ml) fractions of the sucrose gradient were analyzed.

**Table S1. Proteins analyzed in this study**

| Protein | Function | Source organism | Transporter family | Family nr. | Size, kDa |
|---|---|---|---|---|---|
| LacY | Lactose/$H^+$ symporter | *E. coli* | MFS | 2.A.1 | 46.5 |
| GltP | Glutamate/$H^+$ symporter | *E. coli* | DAACS | 2.A.23 | 47.2 |
| EcClC | $Cl^-/H^+$ antiporter | *E. coli* | ClC | 2.A.49 | 50.3 |
| NhaA | $Na^+/H^+$ antiporter | *E. coli* | NhaA | 2.A.33 | 41.4 |
| DctA | Dicarboxylate/$H^+$ symporter | *E. coli* | DAACS | 2.A.23 | 45.4 |
| LacS(ΔIIA) | Lactose/$H^+$ symporter | *S. thermophilus* | GPH (MFS) | 2.A.2 | 52.3 |
| YdjN | Unknown | *E. coli* | DAACS | 2.A.23 | 48.7 |
| SstT | Serine/$Na^+$ symporter | *E. coli* | DAACS | 2.A.23 | 43.5 |
| GlpF | Glycerol facilitator | *E. coli* | MIP | 1.A.8 | 29.8 |

MFS, Major Facilitator Superfamily; DAACS, Dicarboxylate/Amino Acid:Cation ($Na^+$ or $H^+$) Symporter Family; ClC, Chloride Carrier/Channel Family; NhaA, $Na^+$:$H^+$ Antiporter (NhaA) Family; GPH, Glycoside-Pentoside-Hexuronide:Cation Symporter Family; MIP, Major Intrinsic Protein Family. Family names and numbers are derived from the Transport Classification Database [Saier MH, Jr (1988) Molecular phylogeny as a basis for the classification of transport proteins from bacteria, archaea, and eukarya. *Adv Micro Physiol* 40:81–136].